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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 207/34, A61K 31/40 | A1

(11) International Publication Number: WO 97/03957

(43) International Publication Date: 6 February 1997 (06.02.97)

(21) International Application Number: PCT/EP96/02659

(22) International Filing Date: 19 June 1996 (19.06.96)

(30) Priority Data:
9514993.6 21 July 1995 (21.07.95) GB

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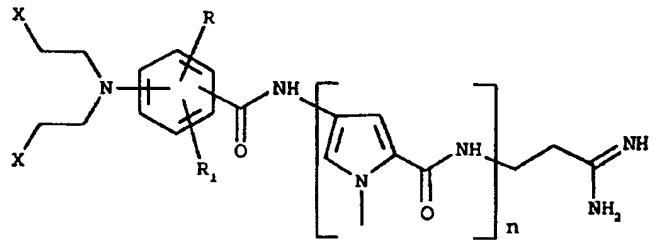
(81) Designated States: AM, AU, BB, BG, BR, BY, CA, CN, CZ, EE, FI, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LV, MD, MG, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, TJ, TM, TT, UA, UG, US, UZ, VN, Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: BIS-(2-HALOETHYL)AMINOPHENYL SUBSTITUTED DISTAMYCIN DERIVATIVES AS ANTITUMOR AND ANTIVIRAL AGENTS



(I)

(57) Abstract

Novel antitumor and antiviral agents of formula (I) wherein n is 2, 3 or 4; one of R and R_1 is hydrogen, C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy and the other is independently CF_3, C_1-C_4 alkyl or C_1-C_4 alkoxy; and X is halogen; and the salts thereof are disclosed.

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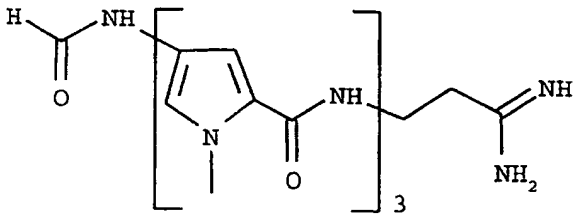
BIS-(2-HALOETHYL)AMINOPHENYL SUBSTITUTED DISTAMYCIN DERIVATIVES AS ANTITUMOR AND ANTIVIRAL AGENTS

The present invention refers to novel antitumor alkylating and antiviral agents related to the known antibiotic distamycin A.



(distamycin A)

which belongs to the family of the pyrroleamidine antibiotics and is reported to interact reversibly and selectively with DNA-AT sequences interfering with both replication and transcription [Nature 203, 1064 (1964); FEBS Letters 7 (1970) 90; Prog. Nucleic Acids Res.Mol.Biol., 15, 285 (1975)].

DE-A-1795539 describes the preparation of distamycin derivatives in which the formyl group of distamycin is replaced by hydrogen or the acid residue of an organic C_1-C_4 aliphatic acid or of cyclopentylpropionic acid.

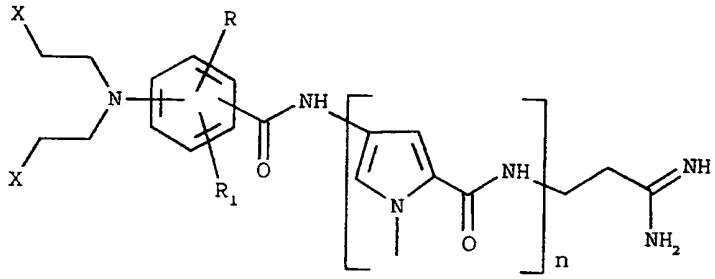
EP-B-246868 describes distamycin A analogs in which the distamycin formyl group is substituted by aromatic, alicyclic or heterocyclic moieties bearing alkylating groups.

It has now been found that a selected class of compounds falling within the general chemical formula of EP-B-246868 has more valuable biological properties than the related prior art compounds.

Accordingly the present invention provides new site specific nitrogen mustards, a process for their preparation, pharmaceutical compositions containing them and their use in

therapy.

The invention herein provides a novel class of compounds of formula (I)



(I)

wherein

n is 2, 3 or 4;

one of R and R_1 is hydrogen, C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy and the other is independently CF_3, C_1-C_4 alkyl or C_1-C_4 alkoxy; and

X is halogen.

The invention includes also the pharmaceutically acceptable salts of the compounds of formula (I) as well as all the possible isomers covered by formula (I), both separately and in mixture.

The present invention also include within its scope both the metabolites and the pharmaceutically acceptable bio-precursors (otherwise known as pro-drugs) of the compounds of formula (I).

The alkyl and alkoxy groups may be branched as straight carbon chains.

A C_1-C_4 alkyl group is preferably methyl or ethyl.

A C_1-C_4 alkoxy group is preferably methoxy or ethoxy.

In the phenyl ring the carbamoyl and the bis-halo-ethylamino

groups are each other preferably in the meta or para positions.

R and R_1 can be on any of the free carbon atoms of the phenyl ring, not on the same carbon atom of course. Preferably one of R and R_1 is hydrogen or C_1-C_4 alkyl and the other is C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy; or R and R_1 are the same and are C_1-C_4 alkoxy.

Pharmaceutically acceptable salts of the compounds of formula (I) are their salts with pharmaceutically acceptable, either inorganic or organic, acids.

Examples of inorganic acids are hydrochloric, hydrobromic, sulfuric and nitric acid; examples of organic acids are acetic, propionic, succinic, malonic, citric, tartaric, methanesulfonic and p-toluenesulfonic acid.

A particularly preferred n value is 3.

X is preferably chloro or bromo, in particular chloro.

A preferred class of compounds according to the present invention are the compounds of formula (I) wherein:

n is 3;

X is chloro;

one of R and R_1 is hydrogen or C_1-C_4 alkyl and the other is C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy; and the pharmaceutically acceptable salt thereof.

Examples of specific compounds under this invention, especially in the form of salts preferably with hydrochloric acid, are the following:

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-dimethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-ethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-diethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methoxy-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-ethoxy-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[2-methoxy-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

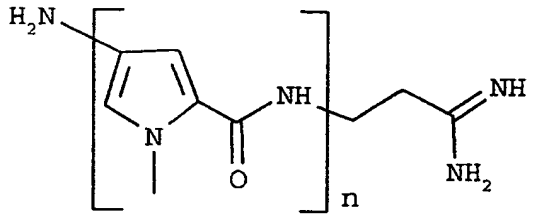
β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[2-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-

carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine; and

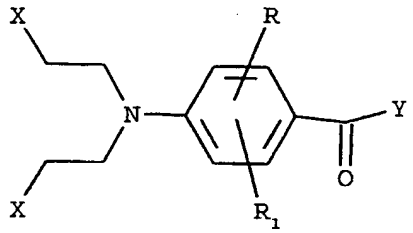
β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-5-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido] pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine.

The compounds of the invention and the salts thereof can be obtained by a process comprising reacting a compound of formula (II)



(II)

wherein n is as defined above, with a compound of formula (III)



(III)

wherein

R, R_1 and X are as defined above and Y is hydroxy or leaving group; and, if desired, salifying a compound of formula (I) or obtaining a free compound from a salt thereof, and/or, if desired, separating a mixture of isomers of a compound of formula (I) into the single isomers.

The reaction of a compound of formula (II) with a compound of formula (III) can be carried out according to known methods,

for instance those described in EP-B-246868.

In particular Y as a leaving group can be a group chosen from halogen, in particular chlorine, 2,4,5-trichlorophenoxy, 2,4-dinitrophenoxy, succinimido-N-oxy and imidazolyl group.

The reaction between a compound of formula (II) and a compound of formula (III) wherein Y is -OH is preferably carried out in a molecular ratio from 1:1 to 1:2 in an organic solvent such as e.g., dimethylsulphoxide, hexamethylphosphotriamide, dimethylacetamide, dimethylformamide, ethyl alcohol, benzene or pyridine, in the presence of an organic or inorganic base such as, e.g., triethylamine, diisopropyl ethylamine or sodium carbonate or bicarbonate, and of a condensing agent such as, e.g., N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide or preferably, N,N'-dicyclohexylcarbodiimide. The reaction temperature may vary from about -10° C to about 50° C and the reaction time from about 1 to about 24 hours.

The reaction between a compound of formula (II) and a compound of formula (III), wherein Y is another leaving group, e.g. halogen, 2,4,5-trichlorophenoxy or succinimido-N-oxy or imidazolyl, may be carried out in analogous conditions but without the condensing agent.

The compounds of formula (II) are known compounds or may be prepared by known methods from known compounds: see, for instance, Arcamone et al., Gazzetta Chim. Ital. 97, 1097 (1967). The compounds of formula (III) are known compounds too or may be prepared from known compounds through reactions well described in the organic chemistry: see for example J.Med. Chem. 9, 882 (1966) and 25, 178 (1982).

The salification of a compound of formula (I) as well as the preparation of a free compound from a salt may be carried out

by known standard methods.

Well known procedures such as, e.g. fractional crystallization or chromatography may also be followed for separating a mixture of isomers of formula (I) into the single isomers.

The new compounds of formula (I) prepared according to the above described procedures may be as well purified by conventional methods such as, e.g., silica gel or alumina column chromatography, and/or by recrystallization from an organic solvent such as, e.g., a lower aliphatic alcohol, e.g. methyl, ethyl or isopropyl alcohol, or dimethylformamide.

PHARMACOLOGY

The compounds of the invention can be useful as antineoplastic and antiviral agents. They show, in particular, cytostatic properties towards tumor cells so that they can be useful, e.g., to inhibit the growth of various tumors, such as, for instance, carcinomas, e.g. mammary carcinoma, lung carcinoma, bladder carcinoma, colon carcinoma, ovary and endometrial tumors in mammals, including humans. Other neoplasias in which the compounds of the invention could find application are, for instance, sarcomas, e.g. soft tissue and bone sarcomas, and the hematological malignancies such as, e.g. leukemias.

The antitumor activity was evaluated in vitro by cytotoxicity studies carried out on murine L1210 leukemia cell. Cells were derived from in vivo tumors and established in cell culture. Cells were used until the tenth passage. Cytotoxicity was determined by counting surviving cells after 48 hours treatment.

The percentage of cell growth in the treated cultures was compared with that of controls. IC_{50} values (inhibiting concentration 50% of the cellular growth in respect to controls) were calculated on dose-response curves.

The compounds of the invention were tested also in vivo on murine L_{1210} leukemia and on murine reticulosarcoma M 5076, showing a very good antitumoral activity, with the following procedure.

L_{1210} murine leukemia was maintained in vivo by i.v. serial transplantation. For experiments, 10^5 cells were injected i.p. in CD2F1 female mice, obtained from Charles River Italy. Animals were 8 to 10 weeks old at the beginning of the experiments. Compounds were administered i.v. at day +1 after tumor cells injections.

M5076 reticulosarcoma was maintained in vivo by i.m. serial transplantation. For experiments, $5x10^5$ cells were injected i.m. in C57B16 female mice, obtained from Charles River Italy. Animals were 8 to 10 weeks old at the beginning of the experiments. Compounds were administered i.v. at day 3, 7 and 11 after tumor injection.

Survival time of mice and tumor growth were calculated and activity was expressed in term of T/C% and T.I.%.

$$T/C = \frac{\text{median survival time treated group}}{\text{median survival time untreated group}} \times 100$$

T.I.= % inhibition of tumor growth respect to control

Tox: number of mice which died for toxicity.

Tox determination was made when mice died before the control and/or tested significant body weight loss and/or spleen and/or liver size reduction were observed.

The compounds of the invention showed higher antitumor activity in these tumor models than closely related compounds known from EP-B-0246868.

For example, the representative compounds β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl) aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine (internal code FCE 29325) and β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-dimethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] pyrrole-2-carboxamido]propionamidine (internal code FCE 29721) and the prior art compound, according to EP-B-0246868, β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido] pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine (internal code FCE 24517), were tested against disseminated L_{1210} murine leukemia showing the following activity data.

Table 1

Compound (internal code)	*mg/kg*	*T/C %*	*Tox*
FCE 29325	3.13	191	0/10
FCE 29721	3.13	183	0/10
FCE 24517	3.13	133	0/10

The activity data occurring in above Table 1 show that the compounds of the instant invention, bearing the claimed substituents on the phenyl ring of the benzoyl mustard moiety, are more active than the closely related unsubstituted prior art compound FCE 24517.

The compounds of the invention show also a remarkable effectiveness in interfering with the reproductive activity

of the pathogenic viruses and protect tissue cells from the viral infections.

For example they show activity against DNA viruses such as, for instance, herpes, e.g. herpes simplex and herpes zoster, viruses, virus vaccinia, RNA viruses such as, e.g. Rhinovirus and Adenoviruses, and against retroviruses such as, for instance, Sarcoma viruses, e.g., Murine sarcoma virus, and Leukemia viruses, e.g. Friend leukemia virus. Thus, for example, herpes, coxsackie and respiratory syncytial viruses were tested in fluid medium as follows. Serial twofold dilutions of the compounds from 200 to 1.5 mcg/ml were distributed in duplicate 0.1 ml/well in 96 wells microplates for tissue culture.

Cell suspensions ($2x10^5$ cells/ml) infected with about $5x10^{-3}$ $TCID_{50}$ of virus/cell were immediately added 0.1 ml/well.

After 3-5 day incubation at 37° C in CO_2 5%, the cell cultures were evaluated by microscopical observation and Minimum Inhibiting Concentration (MIC) were determined, MIC being the minimum concentration which determines a reduction of cytopathic effect in comparison with the infected controls.

The compounds of the invention can be administered to mammals, including humans, by the usual routes, for example, parenterally, e.g. by intravenous injection or infusion, intramuscularly, subcutaneously, topically or orally.

The dosage depends on the age, weight and conditions of the patient and on the administration route.

For example, a suitable dosage for administration to adult humans for the compound FCE 29325 may range from about 0.1 to about 150-200 mg pro dose 1-4 times a day.

As already said, the pharmaceutical compositions of the invention contain a compound of formula (I) as the active substance, in association with one or more pharmaceutically acceptable excipients.

The pharmaceutical compositions of the invention are usually prepared following conventional methods and are administered in a pharmaceutically suitable form.

For instance, solutions for intravenous injection of infusion may contain as carrier, for example, sterile water or preferably, they may be in the form of sterile aqueous isotonic saline solutions.

Suspensions or solutions for intramuscular injections may contain, together with the active compound a pharmaceutically acceptable carrier, e.g. sterile water, olive oil, ethyl oleate, glycols, e.g. propylene glycol, and if desired, a suitable amount of lidocaine hydrochloride.

In the forms for topical application, e.g. creams, lotions or pastes for use in dermatological treatment, the active ingredient may be mixed with conventional oleaginous or emulsifying excipients.

The solid oral forms, e.g. tablets and capsules, may contain, together with the active compound, diluents, e.g., lactose, dextrose, saccharose, cellulose, corn starch and potato starch; lubricants, e.g. silica, talc, stearic acid, magnesium or calcium stearate, and/or polyethylene glycols; binding agents, e.g. starches, arabic gums, gelatin, methylcellulose, carboxymethyl cellulose, polyvinylpyrrolidone; disaggregating agents, e.g. a starch, alginic acid, alginates, sodium starch glycolate; effervescing mixtures; dyestuffs; sweeteners; wetting agents, for instance, lecithin, polysorbates, laurylsulphates; and,

in general, non-toxic and pharmacologically inactive substances used in pharmaceutical formulation. Said pharmaceutical preparation may be manufactured in a know manner, for example by means of mixing, granulating, tabletting, sugar-coating or film-coating processes.

Furthermore, according to the invention there is provided a method of treating tumors and viral infections in a patient in need of it, comprising administering to the said patient a composition of the invention.

A further object of the present invention is a combined method of treatment of cancer or of amelioration of the conditions of mammals, including humans, suffering from cancer, said method comprising administering:

1) a compound of the invention, or a pharmaceutically acceptable salt thereof, and

2) an additional antitumor agent, in amounts and close enough together in time sufficient to produce a therapeutically useful effect.

The present invention also provides products containing a compound of the invention, or a pharmaceutically acceptable salt thereof, and an additional antitumour agent as a combined preparation for simultaneous, separate or sequential use in anti-cancer therapy.

The term "antitumor agent" is meant to comprise both a single antitumor drug and "cocktails" i.e. a mixture of such drugs, according to the clinical practice.

Examples of antitumor agents that can be formulated with a compound of the invention or alternatively, can be administered in a combined method of treatment, include

doxorubicin, daunomycin, epirubicin, idarubicin, etoposide, fluoro-uracil, melphalan, cyclophosphamide, 4-demethoxy daunorubicin, bleomycin, vinblastin and mitomycin or a mixtures of two or more thereof.

The compounds of the invention can therefore be used in a treatment to ameliorate a cancer. They may be administered to a patient suffering from a cancer treatable with an antitumor agent, for example an anthracycline glycoside such as doxorubicin, daunomycin, epirubicin, 4-demethoxy daunorubicin or idarubicin as mentioned above, together with the antitumor agent.

A compound of the invention and an antitumor agent such as an anthracycline glycoside can be administered to improve the condition of a patient having a leukaemia lymphoma, sarcoma, such as myeloblastic leukaemia, neuroblastoma, Wilm's tumor or malignant neoplasm of the bladder, breast, lung or thyroid.

The following examples illustrate but do not limit the invention.

The abbreviations DMF, DMSO and P.M.R. stand for dimethylformamide, dimethylsulfoxide and proton magnetic resonance respectively.

Example 1

The compound β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine hydrochloride

Step one The intermediate 3-methyl-4-N,N-bis(2-chloroethyl) aminobenzylic acid

To a suspension of 2 g of commercial ethyl 3-methyl-4-aminobenzoate in 100 ml of a solution acetic acid 25% were added 20 ml of ethylene oxide. The mixture was stirred at room temperature for two days, neutralized with sodium bicarbonate and extracted with ethyl acetate (2 x 100 ml).

The combined organic phases were dried (Na_2SO_4) and concentrated in vacuo to yield ethyl 3-methyl-4-N,N-bis(2-hydroxy) aminobenzoate as a white precipitate, which was filtered, suspended in 10 ml of a solution of hydrochloric acid 23%, cooled in ice and added of 1.8 ml of phosphorus oxychloride. The mixture was refluxed for two hours, cooled, diluted with water and extracted with ethyl acetate (2 x 50 ml).

The combined organic phases were dried (Na_2SO_4) and solvent evaporated in vacuo to yield 2 g of the intermediate.

m.p. 108 - 110°C

FAB-MS: m/z: 276 (20, $[M+H]^+$)

P.M.R. ($CDCl_3$) δ: 7.9 (m, 2H); 7.15 (m, 1H); 3.5 (m, 8H); 2.35 (s, 3H)

Step two The title compound

To a solution of 630 mg of the intermediate in 10 ml of benzene were added 1.8 ml of thionyl chloride. The mixture was refluxed for two hours, the solvent evaporated in vacuo, the crude solid residue dissolved in 15 ml of dioxane and added in small portions to a solution of 400 mg of N-deformyl distamycin A, 255 mg of sodium bicarbonate in 10 ml of water.

The mixture was stirred for one hour and then added of a solution of hydrochloric acid 2N until pH=1. The solvent was evaporated in vacuo and the solid residue purified by flash chromatography on silica gel with a mixture of methylene chloride, methanol, yielding 500 mg of the title compound.

FAB-MS: m/z: 711 (45[M+H]$^+$), 258 (75)

P.M.R. (DMSO) δ: 10.19 (s, 1H); 9.97 (s, 1H); 9.91 (s, 1H); 8.7 (bs, 4H); 8.21 (t, J=5.7 Hz, 1H); 7.74 (m, 2H); 7.29 (d, J=1.8 Hz, 1H); 7.28 (d, J=7.5 Hz, 1H); 7.22 (d, J=1.8 Hz, 1H); 7.17 (d, J=1.8 Hz, 1H); 7.08 (d, J=1.8 Hz, 1H); 7.05 (d, J=1.8 Hz, 1H); 6.94 (d, J=1.8 Hz, 1H); 3.85 (s, 3H); 3.83 (s, 3H); 3.80 (s, 3H); 3.3-3.7 (m, 10H); 2.6 (t, J=6.6 Hz, 2H); 2.33 (s, 3H).

By analogous procedure and using the opportune intermediate the following compounds can be obtained:

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-dimethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine hydrochloride

FAB-MS: m/z: 725 (90[M+H]$^+$)

U.V. (EtOH 95%) λ 310; ε = 42985

P.M.R. (DMSO) δ: 10.22 (s, 1H); 10.01 (s, 1H); 9.94 (s, 1H); 8.99 (s, 2H); 8.64 (s, 2H); 8.21 (t, J=5.7 Hz, 1H); 7.61 (s, 2H); 7.29 (d, J=1.7 Hz, 1H); 7.21 (d, J=1.7 Hz, 1H); 7.18 (d, J=1.7 Hz, 1H); 7.08 (d, J=1.7 Hz, 1H); 7.05 (d, J=1.7 Hz, 1H); 6.91 (d, J=1.7 Hz, 1H); 3.86 (s, 3H); 3.84 (s, 3H); 3.81 (s, 3H); 3.62 (m, 2H); 3.60-3.30 (m, 8H); 2.62 (m, 2H); 2.35 (s, 6H).

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-ethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-diethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methoxy-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-ethoxy-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[2-methoxy-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[2-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine; and

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-5-

methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido] pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine.

Example 2

Tablets each weighting 0.250 g and containing 50 mg of the active substance can be manufactured as follows:

Composition for 10.000 tablets	
β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido] pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride	500 g
Lactose	1.400 g
Corn starch	500 g
Talc powder	80 g
Magnesium stearate	20 g

The β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis (2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido] propionamidine hydrochloride, the lactose and half the corn starch are mixed; the mixture is then forced through a sieve of 0.5 mm mesh size.

Corn starch (10 g) is suspended in warm water (90 ml) and the resulting paste is used to granulate the powder. The granulate is dried, comminuted on a sieve of 1.4 mm mesh size, then the remaining quantity of starch, talc and magnesium stearate is added, carefully mixed and processed into tablets.

Example 3

Capsules, each dosed at 0.200 g and containing 20 mg of the active substance can be prepared as follows:

Composition for 500 capsules	
β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride	10 g
Lactose	80 g
Corn starch	5 g
Magnesium stearate	5 g

This formulation can be encapsulated in two-piece hard gelatin capsules and dosed at 0.200 g for each capsule.

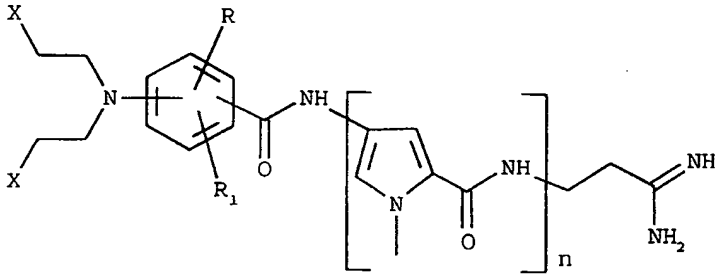
Example 4

Intramuscular Injection 25 mg/ml

An injectable pharmaceutical composition can be manufactured by dissolving 25 g of β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine hydrochloride in sterile propyleneglycol (1000 ml) and sealing ampoules of 1-5 ml.

CLAIMS

1. A compound of formula (I)



(I)

wherein

n is 2, 3 or 4;

one of R and R_1 is hydrogen, C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy and the other is independently CF_3, C_1-C_4 alkyl or C_1-C_4 alkoxy; and

X is halogen and the pharmaceutically acceptable salts thereof.

2. A compound of formula (I), according to claim 1, wherein

n is 3;

X is chloro;

one of R and R_1 is hydrogen or C_1-C_4 alkyl and the other is C_1-C_4 alkyl, CF_3 or C_1-C_4 alkoxy; and the pharmaceutically acceptable salts thereof.

3. A compound selected from:

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]

pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-dimethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-ethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3,5-diethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-methoxy-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-ethoxy-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[2-methoxy-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

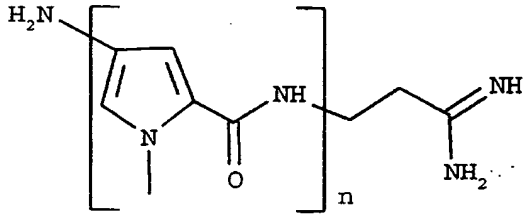
β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[2-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine;

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine; and

β-[1-methyl-4-[1-methyl-4-[1-methyl-4-[3-trifluoromethyl-5-methyl-4-N,N-bis(2-chloroethyl)aminobenzene-1-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]pyrrole-2-carboxamido]propionamidine; or a pharmaceutically acceptable salt thereof.

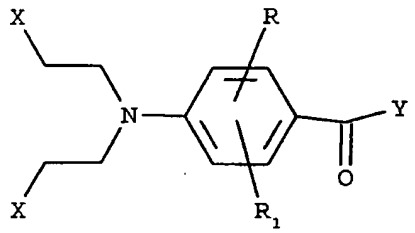
4. A salt of a compound according to claim 3, wherein said salt is the hydrochloride.

5. A process for the preparation of a compound of formula (I), according to claim 1, or a salt thereof, said process comprising reacting a compound of formula (II)



(II)

wherein n is as defined in claim 1, with a compound of formula (III)



(III)

wherein

R, R_1 and X are as defined in claim 1 and Y is hydroxy or leaving group; and, if desired, salifying a compound of formula (I) or obtaining a free compound from a salt thereof, and/or, if desired, separating a mixture of isomers of a compound of formula (I) into the single isomers.

6. A pharmaceutical composition containing a suitable carrier and/or diluent and, as an active principle, a compound of formula (I) according to claim 1 or a pharmaceutically acceptable salt thereof.

7. A compound of formula (I), according to claim 1, or a pharmaceutically acceptable salt thereof, for use as antineoplastic and antiviral agent.

8. Product containing a compound of formula (I), as defined in claim 1, or a pharmaceutically acceptable salt thereof, and an additional antitumour agent as a combined preparation for simultaneous, separate or sequential use in anti-cancer therapy.

9. Use of a compound of formula (I), as defined in claim 1, or a pharmaceutically acceptable salt thereof, in the manufacture of a pharmaceutical composition for use as an antineoplastic and antiviral agent.

10. A method of treating a mammal in need of an antineoplastic agent, the method comprising administering to said mammal a therapeutically effective amount of a compound of formula (I), as defined in claim 1, or a pharmaceutically acceptable salt thereof.

INTERNATIONAL SEARCH REPORT

Inter ·tional Application No
PC1/EP 96/02659

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D207/34 A61K31/40

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. MED. CHEM. (1989), 32(4), 774-8 CODEN: JMCMAR;ISSN: 0022-2623, 1989, XP000608784 ARCAMONE, FEDERICO MARIA ET AL: "Synthesis, DNA-binding properties, and antitumor activity of novel distamycin derivatives" see the whole document ---	1-10
A	EP 0 246 868 A (FARMITALIA CARLO ERBA S.P.A., ITALY) 25 November 1987 cited in the application see the whole document -----	1-10

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 November 1996	19.11.1996

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kissler, B

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

:national application No.
PCT/EP 96/02659

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claim 10 is directed to a method of treatment of (diagnostic method practised on) the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte tional Application No
PCT/EP 96/02659

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0246868	25-11-87	AU-B-	597659	07-06-90
		AU-A-	7316387	26-11-87
		BG-B-	60531	28-07-95
		CA-A-	1314551	16-03-93
		DE-A-	3781716	22-10-92
		HK-A-	31993	08-04-93
		IE-B-	60198	15-06-94
		JP-B-	6023193	30-03-94
		JP-A-	62294653	22-12-87
		KR-B-	9511408	04-10-95
		SU-A-	1528316	07-12-89
		US-A-	5017599	21-05-91
		US-A-	5049579	17-09-91
		US-A-	5310752	10-05-94
		ZA-A-	8703593	12-11-87